Therapeutic applications of ghrelin agonists in the treatment of gastroparesis

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Abstract

There remains an unmet need for effective pharmacologic treatments for gastroparesis. Ghrelin is the endogenous ligand for the growth hormone secretagogue receptor and has been shown to regulate energy homeostasis and exert prokinetic effects on gastrointestinal motility. In recent years, several ghrelin receptor agonists have been studied in clinical trials of patients with diabetic gastroparesis. The intravenous macrocyclic peptidomimetic, TZP-101 initially suggested improvement in gastroparesis symptoms with intravenous administration when compared to placebo. However, in subsequent studies of oral preparations, TZP-102 failed to confirm these results. Another ghrelin receptor agonist, RM-131 was recently shown to significantly accelerate gastric emptying (GE) in patients with type 1 and type 2 diabetes and delayed GE. RM-131 reduced total Gastroparesis Cardinal Symptom Index-Daily Diary (GCSI-DD) and composite scores among type 1 diabetics. Continued development of ghrelin agonists should be explored in attempts to expand therapeutic options for the treatment of gastroparesis.

Introduction

Gastroparesis is a disorder of gastric motility characterized by a constellation of cardinal upper gastrointestinal (GI) symptoms including nausea, vomiting, early satiety or postprandial fullness, bloating, and abdominal pain that occurs in association with delayed gastric emptying (GE) without mechanical obstruction of the stomach [1]. It is a heterogeneous disorder in which different etiologies may impact symptoms or response to treatment. The most common etiologies of gastroparesis include diabetes and postsurgical, followed by neurologic, rheumatologic and other miscellaneous disorders [2]. However, the etiology is unknown in approximately 40% of patients with gastroparesis, a condition identified by idiopathic gastroparesis. Mechanisms that contribute to pathophysiology of gastroparesis are variable, and gastric emptying profiles cannot specifically distinguish neuropathic from myopathic gastroparesis [3]. Other factors that have been implicated in pathogenesis include impaired glycemic control [4], abnormalities of interstitial cells of Cajal (ICCs) [5,6], alterations in immune infiltrate marked by increase in CD45 and CD68 immunoreactivity [5], decreased heme oxygenase-1 [7,8], and loss of neuronal nitric oxide synthase (nNOS) [5,7,9,10].

The clinical impact of gastroparesis is significant; symptoms, particularly pain, are associated with impaired quality of life and increased anxiety or depression [11] while examination of national trends has shown a > 200% increase in overall hospitalizations for gastroparesis [12]. There remains a need for development of effective and targeted pharmacologic treatments.

Prokinetic agents should be considered in the pharmacologic treatment of gastroparesis to improve gastric emptying and symptoms of gastroparesis [13]. Currently, the only FDA-approved prokinetic agent in the U.S. remains metoclopramide [14], a potent central and peripheral dopamine receptor antagonist that exerts its effects through suppression of the central vomiting center and stimulation of gastric motility via receptor-mediated actions resulting in increased gastric tone, intragastric pressure, antroduodenal coordination and accelerated gastric emptying [15,16]. However, it's use is limited to short-term treatment (no greater than 12 weeks) due to CNS side-effects such as anxiety, agitation, abnormal movements, dystonic reactions, somnolence or confusion, and intractable tardive dyskinesia [17,18]. Other agents include domperidone, which is not available in the U.S. but has been made available through a special program by the FDA [13]. Eryrthromycin, a motilin receptor, may also be utilized but tachyphylaxis remains a limiting factor in its long-term use [17]. More recently, the 28 amino acid residue peptide, ghrelin, has been identified as a potential treatment for impaired gastric emptying due to its actions on intestinal motility and food intake (reviewed in references 19 and 20).

The role of Ghrelin

Originally identified in 1999 as the natural ligand for the growth hormone secretagogue receptor (GHS-1a) or ghrelin receptor (GRLN-R) [19,20], ghrelin is an orexigenic hormone produced mainly in the oxyntic mucosa of the stomach [22] with significant sequence similarity to motilin [23]. It is derived from the human ghrelin gene via alternative splicing [24] and synthesized from precursor peptides that undergo post-translational processing [25]. The majority of circulating ghrelin occurs in the form of deacyl ghrelin which lacks an octanoyl

group that is required for the hormone to be biologically active [26]. Octynoylation at serine-3 of ghrelin is mediated by O-acyltransferase (GOAT), a polytopic membrane-bound enzyme [27] and activation of GRLN-R by biologically active ghrelin results in direct stimulation of pituitary growth hormone (GH) release. Both ghrelin and GRLN-R (a G-protein-coupled receptor) are widely expressed throughout human tissue [19,28]. Thus, in addition to control of GH secretion, ghrelin serves to regulate diverse digestive processes [22], energy homeostasis and food intake [29,30], and numerous other systems (reviewed in 19, 28) including the immune system [31]], reproductive system [32], cardiovascular system [3334], adrenal glands, neoplastic proliferation, and osteoblastic function [35].

Regulation of energy homeostasis by ghrelin has made ghrelin an appealing target for treatment of metabolic disorders including obesity and type 2 diabetes. Ghrelin influences fat deposition [36], induces food intake through peripheral and central mechanisms [37] and appears to be involved in the rewarding properties of food intake through enhancement of hedonic and incentive responses to food-related cues [38]. Fasting expression of ghrelin has been shown to be increased by a high fat diet in a mouse model [39] and in patients with anorexia nervosa and cachexia, plasma ghrelin levels are increased while lower plasma ghrelin levels are observed in obese individuals compared to lean persons or controls [40,41]. Clinical trials in humans conducted with ghrelin in cachexia associated with cancer, cardiac and chronic lung disease have also shown increases in appetite, weight and cardiac output without significant toxicity [42-44].

Effects of ghrelin on gastrointestinal motility

Effects of ghrelin on upper GI functions appear to be mediated through vagal signaling, direct stimulation of the enteric nervous system or via direct effects on the CNS after crossing of the blood-brain barrier [19]. *In vitro* studies have demonstrated presence of GRLN-R in enteric nervous system of human and animal intestinal preparations [45,46]. Enhanced electrical field stimulation (EFS)-induced contractions and a dose-dependent enhancement of the after-contraction evoked during EFS have been observed with administration of ghrelin to rodent stomach strips [45,47,48] via effects that appear to be mediated via cholinergic and tachynergic pathways [47,49,50]. Prokinetic effects of ghrelin on GI transit *in vivo* in rodents have been demonstrated with various routes of administration [reviewed in reference 19] in post-surgical, opioid-induced, and diabetic models. Administration of acylated ghrelin or ghrelin potentiators improved the delayed GE and decreased antral motility in mice exposed to restraint stress [51].

The role of ghrelin on the migratory motor complex (MMC) has also been studied in several animal models to show stimulation of phase II of the MMC via the vagus nerve in the house musk shrew, *Suncus murinus* [52] and induction of a fasted motor pattern, enhancing motility of the antrum and duodenum in the fed or fasted state of healthy rodents [53-56]. Administration of ghrelin and the endogenous acyl-ghrelin potentiator, rikkunshito, restored fed-like motor activities to fasted activities in fenfluramine-treated rats and in a cancer anorexia-cachexia animal model [57]. Meanwhile, consistent reports of effects of ghrelin on colonic contractility have been lacking despite tissue expression of GRLN-R [58-62]. Animal studies in dogs and rodents have shown no effect on colonic motility with intravenous administration of ghrelin, while central or intraperitoneal administration has demonstrated stimulation of colonic motility reflected by decreased colonic transit time and increased intracolorectal pressures and

fluid output [63-67], suggesting that effects of ghrelin or ghrelin agonists on colonic motility are centrally mediated, requiring crossing of the blood brain barrier.

Studies in healthy human volunteers have shown induction of a premature phase III (MMC) using a pharmacologic dose of ghrelin [68] and inhibition of gastric accommodation when ghrelin was administered at higher doses was observed by Ang et al., but this was not associated with an increase in upper GI symptoms or satiety [69]. Cremonini et al. showed no effect on GE of solids or gastric accommodation by single photon emission CT (SPECT) using an intravenous bolus synthetic human ghrelin (0.33 μg/kg) that stimulated GH within the physiologic range of humans [71]. Meanwhile dosing administration of ghrelin with an infusion rate of 10 pmol/kg·min demonstrated acceleration in GE in normal humans in a separate study by Levin et al [72].

Current clinical trials of ghrelin agonists in gastroparesis

Due to its role in modulation of energy homeostasis and GI motility, ghrelin has been identified as a potential treatment for gastroparesis. Abnormal responses to sham feeding with impaired increases in systemic ghrelin have been observed in patients with diabetic and postsurgical gastroparesis when compared to idiopathic gastroparesis and normal controls [72], suggesting that alterations in ghrelin may play a role in gastroparesis. In a randomized, double-blind, cross-over study, administration of a ghrelin infusion increased GE of a 330 kcal rice-pudding test meal in seven of ten patients with diabetic gastroparesis and this effect was independent of vagal tone [73]. Similar results have been found in a study of six patients with idiopathic gastroparesis with enhanced GE and decreased meal-related symptom scores [74] as

well as patients with neurogenic gastroparesis using bolus doses of 1 or 4 μg/kg of ghrelin to suggest extravagal prokinetic actions of ghrelin [75].

Although the potential for ghrelin as a prokinetic agent in the treatment of GI motility disorders such as gastroparesis is promising, it is limited by its short half-life [76] and plasma instability. Consequently, several synthetic ghrelin agonists have been developed and investigated for clinical use. TZP-101 (ulimorelin) is a first-in-class macrocyclic ghrelin analogue with potent binding affinity for the ghrelin receptor [77]. Initial investigations of this agent in patients with diabetic gastroparesis were promising with 20% reduction in GE half-time of solids vs. placebo in a proof of concept study with a trend towards decreased overall postprandial symptoms and postprandial fullness (Table 1) [78]. A phase 2 multicenter dose-ranging study in patients with moderate to severe symptomatic diabetic gastroparesis subsequently demonstrated significant improvements from baseline on day 4 in Gastroparesis Cardinal Symptom Index (GCSI) loss of appetite and vomiting scores with the 80 μg/kg TZP-101 dose when compared to placebo and a 25% improvement in GE half-time for a small subset of combined TZP-101 dose groups compared to a 8% improvement with placebo, although differences in GE $T_{1/2}$ were not statistically significant (Table 1) [79]. *Post-hoc* analysis of a subset of patients with severe nausea and vomiting (baseline severity score of $\geq$3.5 on the GCSI Nausea/Vomiting subscale) enrolled in this phase 2 study found that nausea and vomiting at the end of treatment (day 4) were significantly reduced for the 80 μg/kg TZP-101 dose compared to placebo and at 30 day follow-up, the improvement of nausea and vomiting appeared to persist for the 80 μg/kg and all TZP-101 dose groups (figure 1), although findings did not reach statistical significance (Table 1) [80]. No significant safety issues were identified in either the multicenter

study or subset population, demonstrating TZP-101to be safe and well tolerated with a relatively benign side effect profile [78-80].

Following TZP-101, oral TZP-102 was developed as a unique synthetic ghrelin receptor agonist with a prolonged half-life and promotility activity demonstrated in a rat model of GE with dose-dependent increases in GE of up to 51% compared to vehicle. In a phase 2a clinical study, 92 patients with diabetic gastroparesis were randomized to once-daily TZP-102 (10, 20, 40 mg) or placebo. Significant diabetic gastroparesis symptom improvements were observed in the GCSI for all TZP-102 doses compared to placebo. In contrast, no significant improvements were observed in GE $T_{1/2}$, the primary efficacy endpoint, with any dose (Table 1) [81]. Two phase 2b double-blind, randomized, placebo-controlled clinical trials (studies TZP-102-CL-G003 and TZP-102-CL-G004) were subsequently conducted to evaluate 12 weeks of once daily and three times daily administration of oral TZP-102 in patients with diabetic gastroparesis with the primary outcome measure being average change from baseline through end-of treatment in Daily Diary of Gastroparesis Symptoms Questionnaire (GSDD). Prior results could not be confirmed in these investigations, as improvement in the GSDD were observed in all treatment arms, with no significant difference between intervention and placebo. GE analysis showed no significant differences in change-from-baseline in Week 12 with treatment or placebo (Table 1). Study TZP-102-CL-G004 was prematurely stopped due to lack of efficacy in patients with diabetic gastroparesis [82].

Several explanations for the observed lack of efficacy and conflicting results between the phase 2a and 2b studies have been proposed: (a) a larger than anticipated placebo-response that was more than double that originally anticipated, (b) variations in study design including

differences in breath test methods (6h 13C-octanoate testing in the phase 2a study and 3h 3C-*Spirulina platensis* testing in the phase 2b study) and diabetes type between the two studies, (c) presence of confounding by use of concomitant medications by study participants, (d) a 4-week vs. 12-week study duration with lack of durability assessment in the phase 2a study [82,83]. Nevertheless, the bottom line that neither study showed efficacy with regards to the primary study endpoint remains the same and in conclusion, therapeutic benefits of treatment of TZP-102 could not be demonstrated.

RM-131 is a novel pentapeptide ghrelin receptor agonist with 100-fold more potency than human ghrelin in reversing gastric ileus in animal [84]. In pre-clinical studies prior to acquisition of RM-131 by Rhythm Pharmaceuticals, RM-131 has been shown to cause a dose-dependent increase in food intake and weight gain (both fat and lean mass) with greater potency than human ghrelin [85]. In a rat models, RM-131 was successful in reversing postsurgical, opiate-induced gastric ileus while in normal, nonsurgical primates, RM-131 increased the GE rate [86]. RM-131 also displayed anti-inflammatory effects and increased survival in models of inflammatory bowel disease. In dogs and rodents, treatment with RM-131 showed transient increased in GH levels that returned to baseline with continued administration [85].

The first clinical investigation of RM-131 was performed in women with type 2 diabetes and documentation of delayed GE and gastroparesis symptoms to assess pharmacodynamics and pharmacokinetic profiles, safety and tolerability of a single dose of RM-131. Results showed that compared with placebo, a single subcutaneous injection of RM-131 significantly increased GE $T_{1/2}$ of solids. Improvements in in GE T_{lag}, GE $T_{1/2}$ liquids, and colonic filling at 6 hours were observed, although not statistically significant. No significant effects was observed for total

GCSI-DD score or composite scores for nausea, bloating postprandial fullness and pain; however, the study was not powered for these endpoints and baseline symptom severity was not a criterion for study eligibility [86].

A separate study of similar design conducted among patients with type 1 diabetes and prior documentation of delayed GE demonstrated that treatment with RM-131 resulted in significantly increased early phase GE with decreased gastric retention of solids at 1 hour ($p= 0.005$) and 2 hours ($p=0.019$); however no significant improvement in the primary end point, GE $T_{1/2}$ was observed, possibly due to the small sample size and relatively normal GE $T_{1/2}$ observed in a number of the patients at baseline. Unlike the previous study in patients with type 2 diabetes, symptom assessment showed significant reductions in total GCSI-DD and composite nausea, vomiting, fullness and pain scores for RM-131 when compared with placebo [87].

RM-131 was found to be safe and well tolerated in both trials, with no serious adverse events reported and no clinically significant impact on physical examination, ECG parameters, vital signs, or routine hematology and chemistry laboratory tests [86,87]. Although results of initial clinical trials with RM-131 have been promising, larger scale studies will be required to address generalizability and establish medium and long-term efficacy of this agent. Currently, phase 2 studies are underway among patients with type 1 and type 2 diabetes mellitus and gastroparesis to evaluate effects of multiple dosing regimens of RM-131 on gastric emptying and gastroparesis symptoms as well as overall safety and tolerability.

Conclusion

Modulation of ghrelin receptors with novel ghrelin agonists has been identified as a pathway for new therapeutic options for the treatment of GI motility disorders such as gastroparesis. Prokinetic effects of ghrelin on gastrointestinal motility have been demonstrated in through investigation of ghrelin in animal models and human studies of health and diseased states leading to clinical trials of several ghrelin receptor agonists in the treatment of gastroparesis. The intravenous macrocyclic peptidomimetic, TZP-101 showed initial promise; however, efficient acceleration of GE was not clearly shown [78] and a subsequent oral preparation of TZP-102 [81] was unable to demonstrate significant improvements in symptoms or GE over placebo and thus further study was stopped due to futility. Lack of efficacy of TZP-102 has brought into question the utility of ghrelin receptor agonists as a useful treatment for gastroparesis [88]. However, termination of further investigation of ghrelin agonists would be premature as another agent, RM-131 demonstrated efficacy in small clinical trials of patients with diabetic gastroparesis with respect to GE endpoints and upper GI symptoms based on validated GE assessment by radioscintigraphy and patient-response outcomes by the gastrointestinal cardinal symptom index daily diary (GSCI-DD) [89]. These findings will require further validation in larger clinical trials to assess responses to multiple dosing regimens of longer duration in relevant patient populations.

Other points to consider with respect to development of ghrelin agonists are that clinical efficacy of these agents may be achieved through effects on alternative mechanisms aside from improvement in delayed GE that may be contributing to the pathogenesis of gastroparesis, particularly given the known lack of symptom correlation and GE [90]. Future studies of ghrelin agonists in the treatment of gastroparesis will also require assessment of effects on other gastric

motor functions such as proximal gastric tone and gastric accommodation [69] which may accompany changes in GE. Efficacy in the treatment of gastroparesis may also be associated with the known antiemetic activity of ghrelin [91-93] and/or its effect on appetite and food intake [57,94]. Of note, “hunger” was reported more frequently among patients with type 1 diabetes with RM-131 administration [87]. In addition, responsiveness to treatment with ghrelin agonists among patients with gastroparesis may vary depending on the underlying etiology of the disease and further study among patients with post-surgical and idiopathic gastroparesis will need to be explored. Although actions of ghrelin appear to be mediated mainly through vagal signaling or directly via the enteric nervous system [19], extravagal effects of ghrelin on gastric motility have been show in patients with neurogenic causes of gastroparesis [75]. Furthermore, it has been suggested that there exists potential for desensitization of the ghrelin receptor with repeated dosing; however, in a study of co-administration of acylated and unacylated ghrelin no tachyphylaxis was observed with repeated administration in inducing decreased insulin concentrations [95].

In summary, ghrelin agonists remain an important area of ongoing clinical investigation in the treatment of gastroparesis. Potential therapeutic application of ghrelin and ghrelin agonists remain broad and its effects on gastric motility remain incompletely understood. Early clinical trials in small patient populations have shown promising results that will require further validation before widespread clinical use.

Compliance with Ethics Guidelines

Conflict of Interest

Andrea Shin has participated in past clinical research trials supported by Rhythm Pharmaceuticals

John M. Wo has performed clinical research trial supported by Tranzyme, Inc, GlaxoSmithKline, and Theravance.
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Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

Andrea Shin has been involved in two clinical trials involving human subjects (see references 86 and 87). Both trials were performed with approval by the Mayo Clinic Institutional Review Board after signed written informed consent and confirmation of patient study eligibility.

John M. Wo has been involved in clinical trials involving human subjects (see references 79, 80, 81 and 82). Trials were performed with approval by the University of Louisville Institutional Review Board after signed written informed consent and confirmation of patient study eligibility.

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* Ejskjaer N, Wo JM, Esfandyari T, Mazen Jamal M, Dimcevski G, Tarnow L et al. A phase 2a, randomized, double-blind 28-day study of TZP-102 a ghrelin receptor agonist for diabetic gastroparesis. Neurogastroenterol Motil. 2013;25:e140-50. doi: 10.1111/nmo.12064.

Phase 2a clinical trial of orally administered synthetic ghrelin-receptor agonist, TZP-102 shows improvement in gastroparesis symptoms among patients with diabetic gastroparesis, but is unable to display significant improvement in GE $T_{1/2}$.

* McCallum RW, Lembo A, Esfandyari T, Bhandari BR, Ejskjaer N, Cosentino C et al; TZP-102 Phase 2b Study Group. Phase 2b, randomized, double-blind 12-week studies of TZP-102, a ghrelin receptor agonist for diabetic gastroparesis. Neurogastroenterol Motil. 2013;25:e705-717. doi: 10.1111/nmo.12184.

Phase 2b clinical trial of ghrelin-receptor agonist, TZP-102, in patients with diabetic gastroparesis is unable to confirm prior results of significant improvement in gastroparesis symptoms and is again, unable to demonstrate significant improvement in gastric emptying. The trial is stopped due to futility.

* Van der Ploeg L, Laken H, Sharma S, Datta R, Halem H, Dong J et al. Preclinical gastrointestinal prokinetic efficacy and endocrine effects of the ghrelin mimetic RM-131. Life Sci. 2014;109:20-29.

Pentapeptide RM-131, a novel ghrelin receptor agonist, is studied in animal models to show enhancement of gastric emptying in rodent models of ileus, > 100-fold potency vs. human ghrelin, and transient increases in growth hormone that return to baseline with chronic administration.

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Table 1: Recent clinical trials of synthetic ghrelin agonists in patients with diabetic gastroparesis

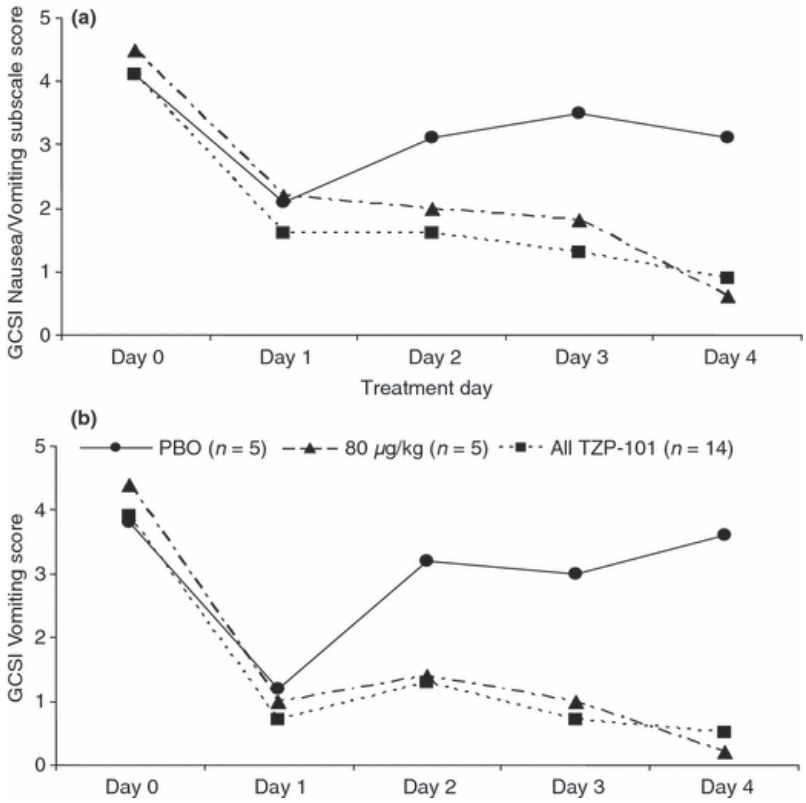
Study	Intervention	Symptom Assessment	p-value	Gastric Emptying	p-value
Ejskjaer et al. 2009 [78]	TZP-101	24% decrease in PP symptom intensity and 37% decrease in PP fullness with TZP-101	ns	20% reduction in solid GE $T_{1/2}$ with TZP-101 vs. pcbo	0.043
Ejskjaer et al. 2010 [79]	TZP-101	Mean change from baseline in GCSI loss of appetite (-2.5 TZP-101, -1.6 pcbo) and vomiting (-2.2 TZP-101, -1.0 pcbo) at day 4 (secondary endpoint)	p=0.034; p=0.006	25% change in GE $T_{1/2}$ with TZP-101 (subset) vs. 8% improvement with pcbo	ns
Wo et al. 2011 [80]	TZP-101 (80 μ/kg)	Change in mean GCSI Nausea/Vomiting and Vomiting at day 4 reduced with TZP-101 vs. pcbo	p<0.001; p=0.008	NA	NA
Ejskjaer et al. 2013 [81]	TZP-102	Improvement in mean GSCI total score (-1.2 with TZP-102; -0.6 with pcbo)	p=0.02	No difference in GE $T_{1/2}$ for TZP-102 vs. pcbo	ns
McCallum et al. 2013 [82]	TZP-102	Improvement in GSDD seen in all treatment arms	ns	No significant difference in change from baseline with TZP-102 or pcbo	ns
Shin et al. 2013 [86]	RM-131	No significant effects for total GCSI-DD score or composite scores	ns	Faster GE $T_{1/2}$ solids with RM-131 (59.5$\pm$7.9) vs. pcbo (127.8$\pm$18.6)	0.011
Shin et al. 2013 [87]	RM-131	Total GCSI-DD score 0.17 on RM-131 and 0.79 on pcbo; lower NVFP composite scores with RM-131	p=0.041; p=0.041	Faster GE solids at 1 and 2 h with RM-131 vs. pcbo.	p=0.005 p=0.019

PP=postprandial; pcbo=placebo; GCSI=Gastroparesis Cardinal Symptom Index; GSDD=Daily Diary of Gastroparesis Symptoms Questionnaire; NVFP=nausea, vomiting, fullness, pain

Figure Legend

Figure 1: Change in mean Nausea/Vomiting subscale scores (a) and Vomiting scores (b) over time. The slopes for days 1–4 in (a) are significantly different for the 80 μg/kg group vs. placebo (P<0.001) and All TZP-101 vs. placebo (P=0.004), and in (b) for 80 μg/kg group vs. placebo (P=0.008) and All TZP-101 vs. placebo (P= 0.005).

Figure 1


(a)
GCSI Nausea/Vomiting subscale score
5
4
3
2
1
0
Day 0
Day 1
Day 2
Day 3
Day 4
Treatment day
(b)
PBO (n = 5)
80 μg/kg (n = 5)
All TZP-101 (n = 14)
GCSI Vomiting score
5
4
3
2
1
0
Day 0
Day 1
Day 2
Day 3
Day 4